## WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



# INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

C07K 14/47, 17/00, 16/18, 16/40, C12N 15/12, 15/61, 15/64, 5/10, 1/19, 1/21, G01N 33/566  (21) International Application Number: PCT/US95/01721 (81) Designated States: CA, JP, US, European patent (AT, BE, C DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE (22) International Filing Date: 10 February 1995 (10.02.95)  (30) Priority Data: 197,795 15 February 1994 (15.02.94) US  (60) Parent Application or Grant (63) Related by Continuation US 197,795 (CON) Filed on 15 February 1994 (15.02.94)  (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).  (72) Inventors; and (75) Inventors/Applicants (for US only): WIEDERRECHT, Gregory, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).  (74) Common Representative: MERCK & CO., INC.; Patent	(51) International Patent Classification 6:		(11) International Publication Number: WO 95/21861
(22) International Filing Date: 10 February 1995 (10.02.95)  (30) Priority Data:	C07K 14/47, 17/00, 16/18, 16/40, C12N 15/12, 15/61, 15/64, 5/10, 1/19, 1/21,	A1	(43) International Publication Date: 17 August 1995 (17.08.95)
(30) Priority Data: 197,795 15 February 1994 (15.02.94) US  (60) Parent Application or Grant (63) Related by Continuation US Filed on 15 February 1994 (15.02.94)  (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).  (72) Inventors; and (75) Inventors/Applicants (for US only): WIEDERRECHT, Gregory, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SEWELL, Tonya, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).	(21) International Application Number:		DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, P1, SE).
(63) Related by Continuation US Filed on 197,795 (CON) Filed on 15 February 1994 (15.02.94)  (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).  (72) Inventors; and (75) Inventors/Applicants (for US only): WIEDERRECHT, Gregory, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SEWELL, Tonya, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).	(30) Priority Data:		Published With international search report.
CO., INC. [US/US]; 126 East Lincoln Avenue, Ranway, NJ 07065 (US).  (72) Inventors; and (75) Inventors/Applicants (for US only): WIEDERRECHT, Gregory, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SEWELL, Tonya, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).	(63) Related by Continuation US 197,79		
(75) Inventors/Applicants (for US only): WIEDERRECHT, Gregory, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SEWELL, Tonya, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).	CO., INC. [US/US]; 126 East Lincoln Avenue, Ranway, NJ		% & &
(74) Common Representative: MERCK & CO., INC.; Patent	(75) Inventors/Applicants (for US only): WIEDERREC gory, J. [US/US]; 126 East Lincoln Avenue, Ra 07065-0907 (US). SEWELL, Tonya, J. [US/US];	inway,	NJ
Dept., 126 East Lincoln Avenue, Rahway, NJ 07065 (US).	(74) Common Representative: MERCK & CO., INC Dept., 126 East Lincoln Avenue, Rahway, NJ 076	C.; Pat 065 (U	ent (i).

### (54) Title: FK-506 CYTOSOLIC BINDING PROTEIN

#### (57) Abstract

A new homogeneous cytosolic binding protein (FKBP12.6), having a specific binding activity of about 4.8 mg FK-506 per mg protein and a molecular weight of about 10-12 kilodaltons, reversibly binds the immunosuppressant FK-506, but not cyclosporine A (CSA). The protein is unstable to heating at 56 °C for 30 minutes losing its FK-506 binding affinity. The FKBP12.6 protein is isolated from the cytosol of mammalian tissues, preferably bovine or human brain tissue, and can be used in diagnostic and purification procedures involving FK-506-type macrolide immunosuppressants. The FKBP12.6 protein also has peptidyl-proline isomerase enzymatic activity, catalyzing the cis-trans isomerization of proline-containing peptide bonds. In addition, FKBP12.6 binds to and inhibits the phosphatase calcineurin in the presence of FK-506 presence of FK-506.